PCT/● 03/00583

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Patentanmeldung Nr.	Patent application No.	Demande de brevet n°

02078358.5

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Anmeldung Nr:
Application no.: 02078358.5
Demande no:

Anmeldetag:
Date of filing: 14.08.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
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Use of genes identified to be involved in tumor development for the development
of anti-cancer drugs and diagnosis of cancer

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)
revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A61K39/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

USE OF GENES IDENTIFIED TO BE INVOLVED IN TUMOR DEVELOPMENT FOR THE DEVELOPMENT OF ANTI-CANCER DRUGS AND DIAGNOSIS OF CANCER

The present invention relates to the use of the murine genes identified by retroviral insertional tagging as well as their human homologues for the identification and development of anti-cancer drugs, like small molecule

5 inhibitors, antibodies, antisense molecules, RNA interference (RNAi) molecules and gene therapies against these genes and/or their expression products, and especially anti-cancer drugs effective against solid tumors of e.g. lung, colon, breast, prostate, ovarian, pancreas and leukemia. The

10 invention further relates to the use of said genes for the diagnosis of cancer, the use of antibodies or fragments derived therefrom for the diagnosis of cancer, pharmaceutical preparations comprising one or more of said inhibitors and methods for the treatment of cancer using said pharmaceutical

15 preparations.

After a quarter century of rapid advances, cancer research has generated a rich and complex body of knowledge revealing cancer to be a disease involving dynamic changes in the genome. Cancer is thought to result from at least six

20 essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (anti-growth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue

25 invasion and metastasis.

In general, these essential alterations are the result of mutations in genes involved in controlling these cellular processes. These mutations include deletions, point mutations, inversions and amplifications. The mutations

result in either an aberrant level, timing, and/or location of expression of the encoded protein or a change in function of the encoded protein. These alterations can affect cell physiology either directly, or indirectly, for example via
5   signalling cascades.

Identifying genes which promote the transition from a normal cell into a malignant cell provides a powerful tool for the development of novel therapies for the treatment of cancer.

10   One of the most common therapies for the treatment of cancer is chemotherapy. The patient is treated with one or more drugs which function as inhibitors of cellular growth and which are thus intrinsically toxic. Since cancer cells are among the fastest growing cells in the body, these cells
15   are severely affected by the drugs used. However, also normal cells are affected resulting in, besides toxicity, very severe side-effects like loss of fertility.

Another commonly used therapy to treat cancer is radiation therapy. Radiotherapy uses high energy rays to
20   damage cancer cells and this damage subsequently induces cell cycle arrest. Cell cycle arrest will ultimately result in programmed cell death (apoptosis). However, also normal cells are irradiated and damaged. In addition, it is difficult to completely obliterate, using this therapy, all tumor cells.
25   Importantly, very small tumors and developing metastases cannot be treated using this therapy. Moreover, irradiation can cause mutations in the cells surrounding the tumor which increases the risk of developing new tumors. Combinations of both therapies are frequently used and a subsequent
30   accumulation of side-effects is observed.

The major disadvantage of both therapies is that they do not discriminate between normal and tumor cells.

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Furthermore, tumor cells have the tendency to become
resistant to these therapies, especially to chemotherapy.

Therapies directed at tumor specific targets would
increase the efficiency of the therapy due to i) a decrease
5   in the chance of developing drug resistance, ii) the drugs
used in these tumor specific therapies are used at much lower
concentrations and are thus less toxic and iii) because only
tumor cells are affected, the observed side-effects are
reduced.

10   The use of tumor specific therapies is limited by the
number of targets known. Since tumors mostly arise from
different changes in the genome, their genotypes are variable
although they may be classified as the same disease type.
This is one of the main reasons why not a single therapy
15   exists that is effective in all patients with a certain type
of cancer. Diagnosis of the affected genes in a certain tumor
type allows for the design of therapies comprising the use of
specific anti-cancer drugs directed against the proteins
encoded by these genes.

20   Presently, only a limited number of genes involved in
tumor development are known and there is a clear need for the
identification of novel genes involved in tumor development
to be used to design tumor specific therapies and to define
the genotype of a certain tumor.

25   In the research that led to the present invention, a
number of genes were identified by proviral tagging to be
involved in tumor development. Proviral tagging is a method
that uses a retrovirus to infect normal vertebrate cells.
After infection, the virus integrates into the genome thereby
30   disrupting the local organization of the genome. This
integration is random and, depending on the integration site,
affects the expression or function of nearby genes. If a gene
involved in tumor development is affected, the cell has a

selective advantage to develop into a tumor as compared to the cells in which no genes involved in tumor development are affected. As a result, all cells within the tumor originating from this single cell will carry the same proviral

5   integration. Through analysis of the region nearby the retroviral integration site, the affected gene can be identified. Due to the size of the genome and the total number of integration sites investigated in the present invention, a gene that is affected in two or more independent

10 tumors must provide a selective advantage and therefore contribute to tumor development. Such sites of integration are designated as common insertion sites (CIS).

The genes claimed in the present invention are all identified by common insertion sites and are so far not

15 reported to be involved in tumor development. The novel cancer genes that were identified in this manner are the following: *Adam11, AI462175, Cd24a, Edg3, Itgp, Kcnj16, Kcnk5, Kcnn4, Ltb, Ly108, Ly6i*, mouse homologue of *EMILIN, Mrc1, Ninj2, Nphs1, Sema4b, Tm9sf2*, and *Tnfrsf17*, encoding

20 cell surface proteins; *Apobec2, Btd, Cds2, Clpx, Ddx19, Ddx21, Dnmt2, Dqx1, Hdac7a, Lce-pending, Mgat1*, mouse homologue of *CILP*, mouse homologue of *NOH61, Nudel-pending, Pah, Pdi1, Ppia, Prps1, Ptgds*, and *Vars2*, encoding enzymes; *Dagk4*, mouse homologue of *PSK, Nme2, Snf11k*, and *Tyki*,

25 encoding kinases; *Dusp10, Inpp4a, Inpp5b, Pps, Ptpn1*, and *Ptpn5*, encoding phosphatases; *Il16, Prg*, and *Scya4*, encoding secreted factors; *Akap7, Api5, Arfrp1, Arhgap14-pending, Cish2, Dapp1, Fabp6, Fkbp8, Fliz1-pending, Hint, Ier5, Jundp2-pending, Lmo6, Mid1*, mouse homologue of *AKAP13*, mouse

30 homologue of *BIN2*, mouse homologue of *CEZANNE*, mouse homologue of *CHD2*, mouse homologue of *MBLL*, mouse homologue of *SLC16A10*, mouse homologue of *SLC16A6*, mouse homologue of *SLC17A5*, mouse homologue of *TAF5L*, mouse homologue of

U1SNRNPBP, mouse homologue of *ZNF8*, *Mtap7*, *Myo1c*, *Nkx2-3*, *Nsf*, *Pcdh9*, *Pkig*, *Prdx2*, *Pscd1*, *Psmb1*, *Psme1*, *Psme2*, *Rgl1*, *Ril-pending*, *Sax1*, *Slc14a2*, *Slc7a1*, *Slc7a11*, *Swap70*, *Txnip*, and *Ubl3*, encoding signaling proteins; *Clic3*, *Gtl1-13*, mouse

5 homologue of *NOL5A*, and *Vdac2*, encoding structural proteins; *ABT1-pending*, *Ctbp1*, *Dermo1*, *Ebf*, *Elf4*, *Ldb1*, mouse homologue of *NR1D1*, mouse homologue of *ZER6*, *Rest*, *Tbp*, *Yy1*, *Zfp238*, *Zfp287*, and *Zfp319*, encoding proteins involved in transcriptional regulation; *Lrrc2*, *Satb1*, *Slfn4*, genes with

10 the following Celera identification codes mCG10290, mCG10613, mCG11234, mCG11325, mCG11355, mCG11803, mCG11817, mCG12566, mCG12630, mCG12824, mCG13346, mCG14143, mCG14155, mCG14342, mCG15141, mCG15321, mCG16761, mCG16858, mCG17127, mCG17140, mCG17142, mCG17547, mCG17569, mCG17751, mCG17799, mCG17802,

15 mCG17918, mCG18034, mCG1850, mCG18663, mCG18737, mCG20276, mCG20905, mCG20994, mCG21403, mCG21505, mCG21529, mCG21530, mCG21803, mCG22014, mCG22045, mCG22386, mCG2258, mCG22772, mCG23032, mCG23035, mCG23069, mCG23075, mCG23120, mCG2543, mCG2824, mCG2947, mCG3038, mCG3729, mCG3760, mCG50409,

20 mCG50651, mCG5068, mCG5070, mCG51393, mCG52252, mCG52498, mCG53009, mCG53724, mCG55023, mCG55075, mCG55198, mCG55265, mCG55512, mCG56069, mCG56089, mCG56746, mCG57132, mCG57265, mCG57617, mCG57827, mCG58254, mCG58345, mCG5900, mCG5905, mCG59368, mCG59375, mCG59533, mCG59662, mCG59810, mCG59997,

25 mCG60526, mCG60833, mCG61221, mCG61661, mCG61897, mCG61907, mCG61943, mCG62177, mCG62971, mCG63537, mCG63601, mCG64346, mCG64382, mCG64398, mCG65022, mCG65585, mCG65785, mCG66128, mCG66379, mCG66776, mCG66965, mCG7831, mCG7856, mCG8424, mCG9002, mCG9537, mCG9538, mCG9791, mCG9792, mCG9843,

30 mCG9875, mCG9877, and mCG9880. These genes are also listed in Table 1.

Table 1

Nr.	Celera Gene	[Mouse] Gene Symbol	Mouse Gene Name	Human Gene Symbol	Human Gene Name	group
1	mCG7185	Adam11	a disintegrin and metalloprotease domain 11	ADAM11	a disintegrin and metalloprotease domain 11	cell-surface
2	mCG7193	AI462175	expressed sequence AI462175	HRAP?	signal membrane-associated protein	cell-surface
3	mCG1746	cd24a	CD24a antigen	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	cell-surface
4	mCG1389	Edg3	endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	EDG3	endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	cell-surface
5	mCG16856	Itgp	integrin-associated protein	CD47	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	cell-surface
6	mCG51514	Kcnj16	potassium inwardly-rectifying channel, subfamily J, member 16	KCNJ16	potassium inwardly-rectifying channel, subfamily J, member 16	cell-surface
7	mCG5336	Kcnk5	potassium channel, subfamily K, member 5	KCNK5	potassium channel, subfamily K, member 5 (TASK-2)	cell-surface
8	mCG22845	Kcnn4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	KCNN4	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	cell-surface
9	mCG55511	Ltb	lymphotoxin B	LTB	lymphotoxin beta (TNF superfamily, member 3)	cell-surface
10	mCG4420	Ly108	lymphocyte antigen 108	unknown	unknown	cell-surface
11	mCG3704	Ly61	lymphocyte antigen 6 complex, locus I	human homolog of Ly61	human homolog of Ly61	cell-surface
12	mCG53500	mouse homolog of EMILIN	mouse homolog of EMILIN	EMILIN	elastin microfibril interface located protein	cell-surface
13	mCG14190	Mrc1	mannose receptor, C type 1	MRC1	mannose receptor, C type 1	cell-surface
14	mCG5710	Ninj2	ninjurin 2	NINJ2	ninjurin 2	cell-surface
15	mCG5790	Nphs1	nephrosis 1 homolog, nephrin (human)	NPHS1	nephrosis 1, congenital, Finnish type (nephrin)	cell-surface
16	mCG18462	Smarb	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4B	SDA4B	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4B	cell-surface
17	mCG11191	Tmprss?	transmembrane P superfamily member 2	TMPRSS?	transmembrane P superfamily member 1?	enzyme
18	mCG6956	Tnfrsf1?	tumor necrosis factor receptor superfamily, member 1?	TNFRSF1?	tumor necrosis factor receptor superfamily, member 1?	enzyme
19	mCG8013	Apobec3	apolipoprotein B editing complex 3	APOBEC3	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3	enzyme
20	mCG8109	Btd	biotinidase	BTD	biotinidase	enzyme
21	mCG15177	cds2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	CDS2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	enzyme
22	mCG1610	Clpx	caseinolytic protease X (E.coli)	CLPX	ClpX caseinolytic protease X homolog (E. coli)	enzyme
23	mCG10857	Ddx19	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 19	DDX19	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 19 (DBP5 homolog, yeast)	enzyme
24	mCG11315	Ddx21	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21 (RNA helicase II/Gu)	DDX21	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21	enzyme
25	mCG15107	Dnmt2	DNA methyltransferase 2	DNMT2	DNA (cytosine-5-)-methyltransferase 2	enzyme
26	mCG10554	Dpa1	DEAD RNA-dependent ATPase	DOX1	DEAD RNA-dependent ATPase DOX1	enzyme
27	mCG8016	Dpq1	histone deacetylase 7A	RDAC7A	histone deacetylase 7A	enzyme
28	mCG5775	Lce-pending	long chain fatty acyl elongase	LCE	long-chain fatty-acyl elongase	enzyme
29	mCG14414	Mgat1	mannoside acetylglucosaminyltransferase 1	MGAT1	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	enzyme
30	mCG14430	mouse homolog of CILP	mouse homolog of CILP	CILP	cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	enzyme
31	mCG11395	mouse homolog of NOMK1	mouse homolog of NOMK1	NOMK1	putative nucleolar RNA helicase	enzyme
32	mCG??	Mdx1-pending	nuclear distribution gene E-like	MDEG?	LYS1-interacting protein NUDEL endolipopeptidase A	enzyme
33	mCG11229	Pah	phenylalanine hydroxylase	PAH	phenylalanine hydroxylase	enzyme
34	mCG2209	Pdi1	peptidyl arginine deiminase, type 1	PADI1	peptidyl arginine deiminase, type I	enzyme
35	mCG6946	Pcpn1	peptidylprolyl isomerase A	PPIA	peptidylprolyl isomerase A (cyclophilin A)	enzyme
36	mCG17115	Ptgds	prostaglandin D2 synthase synthetase 1	PTGDS	prostaglandin D2 synthase synthetase 1	enzyme
37	mCG19417	Vars2	valyl-tRNA synthetase (21 kDa, brain)	VARS2	valyl-tRNA synthetase 2	enzyme
38	mCG18744	Doko	diacylglycerol kinase, delta (110 kDa)	DOKO	diacylglycerol kinase, theta (110kD)	kinase
39	mCG13930	Dapk1	mouse homolog of PSK	PSK	protein derived STE20-like kinase PSK	kinase
40	mCG13588	mouse homolog of PSK	expressed in non-metastatic cells 2, protein (NM23B) (nucleoside diphosphate kinase)	NME2	non-metastatic cells 2, protein (NM23B) expressed in	kinase
41	mCG1461	Nme2	SNF1-like kinase	SNF1LK	SNF1-like kinase	kinase
42	mCG14356	Sofl1k	thymidylate kinase family LPS-inducible member	human homolog of Tyk1	human homolog of Tyk1	kinase
43	mCG17000	Tyk1	dual specificity phosphatase 10	DUSP10	dual specificity phosphatase 10	phosphatase
44	mCG15971	Dusp10	inositol polyphosphate-4-phosphatase, type I, 107kD	INPP4A	inositol polyphosphate-4-phosphatase, type I, 107kD	phosphatase
45	mCG15074	Inpp4a	inositol polyphosphate-5-phosphatase, 75 kDa	INPP5B	inositol polyphosphate-5-phosphatase, 75kD	phosphatase
46	mCG7353	Inpp5b	putative phosphatase	human homolog of Ppa	human homolog of Ppa	phosphatase
47	mCG10770	Ppa	protein tyrosine phosphatase, non-receptor type 1	PTPN1	protein tyrosine phosphatase, non-receptor type 1	phosphatase
48	mCG10092	Ptpn1	protein tyrosine phosphatase, non-receptor type 5	PTPN5	protein tyrosine phosphatase, non-receptor type 5 (striatum-enriched)	phosphatase
49	mCG3105	Ptpn5	interleukin 16	IL16	interleukin 16 (lymphocyte chemoattractant factor)	secreted
50	mCG8259	Prg	proteoglycan, secretory granule	PRG1	proteoglycan 1, secretory granule	secreted
51	mCG11126	Scya4	small inducible cytokine A4	SCYA4	small inducible cytokine A4	secreted

Table 1 (continued)

Nr.	Colera Gene	MIMouse Gene Symbol	Mouse Gene Name	Human Gene Symbol	Human Gene Name	group
53	mCG9005	Akap7	A kinase (PRKA) anchor protein 7	AKAP7	A kinase (PRKA) anchor protein 7	signaling
54	mCG1059	Api5	apoptosis inhibitory protein 5	API5	apoptosis inhibitor 5	signaling
55	mCG22071	Arfrp1	ADP-ribosylation factor related protein 1	ARFRP1	ADP-ribosylation factor related protein 1	signaling
56	mCG15346	Arhgap14-pending	the GTPase activating protein 14	SH3GAP3	SH3-BDO Rho GTPase-activating protein 3	signaling
57	mCG8796	Clnk2	cytokine inducible SH2-containing protein 2	STATI2	STAT induced STAT inhibitor-2	signaling
58	mCG4112	Dapp1	dual adapter for phosphotyrosine and 3-phosphoinositides 1	DAPP1	dual adapter of phosphotyrosine and 3-phosphoinositides	signaling
59	mCG3102	Fabp6	fatty acid binding protein 6, ileal (gastrotropin)	FABP6	fatty acid binding protein 6, ileal (gastrotropin)	signaling
60	mCG23117	Fkbp8	FK506 binding protein 8 (38 kDa)	FKBP8	FK506 binding protein 8 (38 kDa)	signaling
61	mCG20093	Flii1-pending	human homolog of Flii1-pending		human homolog of Flii1-pending	signaling
62	mCG3142	Hint	histidine triad nucleotide binding protein	HINT	histidine triad nucleotide binding protein 1	signaling
63	mCG3210	Ier3	immediate early response 3	IER3	immediate early response 3	signaling
64	mCG5743	Junqbx2-pending	Jun dimerization protein 2		unknown	signaling
65	mCG3955	Lmo6	LIM only 6	LMO6	LIM domain only 6	signaling
66	mCG50211	Midi	midline 1	MID1	midline 1 (Opitz/BBB syndrome)	signaling
67	mCG15699	mouse homolog of AKAP13	mouse homolog of AKAP13	AKAP13	A kinase (PRKA) anchor protein 13	signaling
68	mCG16913	mouse homolog of BIN1	mouse homolog of BIN1	BIN1	bridging integrator 2	signaling
69	mCG16763	mouse homolog of C6EANNE	mouse homolog of C6EANNE	C6EANNE	cellular zinc finger anti-NF-kappaB cosine	signaling
70	mCG19747	mouse homolog of CHD2	mouse homolog of CHD2	CHD2	chromodomain helicase DNA binding protein 2	signaling
71	mCG4298	mouse homolog of HALL	mouse homolog of HALL	HALL	CHR-type zinc finger protein, similar to D. melanogaster nucleobindi 3	signaling
72	mCG1108	mouse homolog of SLC16A10	mouse homolog of SLC16A10	SLC16A10	solute carrier family 16 (monocarboxylic acid transporters), member 10	signaling
73	mCG19455	mouse homolog of SLC17A6	mouse homolog of SLC17A6	SLC17A6	solute carrier family 16 (monocarboxylic acid transporters), member 6	signaling
74	mCG15233	mouse homolog of SLC17A5	mouse homolog of SLC17A5	SLC17A5	solute carrier family 17 (anion/sugar transporter), member 5	signaling
75	mCG1770	mouse homolog of TAF5L	mouse homolog of TAF5L	TAF5L	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65 kD	signaling
76	mCG1755	mouse homolog of U1SNRNP8P	mouse homolog of U1SNRNP8P	U1SNRNP8P	U1-snRNP binding protein homolog (70kD)	signaling
77	mCG13551	mouse homolog of ZNF9	mouse homolog of ZNF9	ZNF9	zinc finger protein 9 (clone HF.18)	signaling
78	mCG2816	Mcap7	microtubule-associated protein 7	MAP7	microtubule-associated protein 7	signaling
79	mCG10776	Myo1c	myosin Ic	MYO1C	myosin IC	signaling
80	mCG18907	Nras2-3	N-ras transcription factor related, locus 3 (Drosophila)	LOC159271	similar to HOMEOBOX PROTEIN HOX-1.3	signaling
81	mCG10781	Nsf	N-ethylmaleimide sensitive fusion protein	NSF	N-ethylmaleimide-sensitive factor	signaling
82	mCG51109	Pcdh9	protocadherin 9	PCDH9	protocadherin 9	signaling
83	mCG5444	Pkig	protein kinase inhibitor, gamma	PKIG	protein kinase (cAMP-dependent, catalytic) inhibitor gamma	signaling
84	mCG5911	Prdx2	peroxiredoxin 2	PRDX2	peroxiredoxin 2	signaling
85	mCG13996	Pscd1	pleckstrin homology, Sec7 and coiled/coil domains 1	PSCD1	pleckstrin homology, Sec7 and coiled/coil domains 1 (cytohesin 1)	signaling
86	mCG4504	Psmb1	proteasome (prosome, macropain) subunit, beta type 1	PSMB1	proteasome (prosome, macropain) subunit, beta type 1	signaling
87	mCG22040	Psma1	proteasome (prosome, macropain) 28 subunit, alpha	PSME1	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)	signaling
88	mCG2044	Psme2	proteasome (prosome, macropain) 28 subunit, beta	PSME2	proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	signaling
89	mCG11500	Rgl1	ral guanine nucleotide dissociation stimulator-like 1	RGL	ral guanine nucleotide dissociation stimulator-like 1	signaling
90	mCG15700	Rl1-pending	reversion induced LIM gene	RIL	LIM domain protein	signaling
91	mCG15821	Sax1	spinal cord Sax1 homeobox gene	SAX1	apostia staxis 1 (dominant)	signaling
92	mCG15477	Slc14a2	solute carrier family 14 (urea transporter) member 2	SLC14A2	solute carrier family 14 (urea transporter), member 2	signaling
93	mCG12717	Slc7a1	solute carrier family 7 (cationic amino acid transporter, y+ system) member 1	SLC7A1	solute carrier family 7 (cationic amino acid transporter, y+ system) member 1	signaling
94	mCG20789	Slc7a11	solute carrier family 7 (cationic amino acid transporter, y+ system) member 11	SLC7A11	solute carrier family 7 (cationic amino acid transporter, y+ system) member 11	signaling
95	mCG6705	Snap70	SNAP complex protein, 70 kDa	SNAP70	SNAP-70 protein	signaling
96	mCG14853	Tanlp	thioredoxin interacting protein	TXNIP	thioredoxin interacting protein	signaling
97	mCG22718	Ubl3	ubiquitin-like 3	UBL3	ubiquitin-like 3	signaling
98	mCG11751	Clic3	chloride intracellular channel 3	CLIC3	chloride intracellular channel 3	structure
99	mCG11494	Gtl1-13	gene trap locus 1-13	NUP160	nucleoporin 160kD	structure
100	mCG16833	mouse homolog of NOL5A	mouse homolog of NOL5A	NOL5A	nucleolar protein 5A (56kD with KKE/D repeat)	structure
101	mCG7155	Vdac3	voltage-dependent anion channel 3	VDAC3	voltage-dependent anion channel 3	structure
102	mCG7757	Abtl-pending	activator of basal transcription	ABT1	TATA-binding protein-binding protein	transcription
103	mCG2531	Ctbp1	C-terminal binding protein 1	CTBP1	C-terminal binding protein 1	transcription
104	mCG10120	Dermo1	dermis expressed 1	DERMO1	dermis expressed 1	transcription
105	mCG6084	Ebf	early B-cell factor	EBF	early B-cell factor	transcription
106	mCG5050	Elf4	E74-like factor 4 (ets domain transcription factor)	ELF4	E74-like factor 4 (ets domain transcription factor)	transcription
107	mCG10284	Ldb1	LIM domain binding 1	LDB1	LIM domain binding 1	transcription
108	mCG15540	mouse homolog of NR1D1	mouse homolog of NR1D1	NR1D1	nuclear receptor subfamily 1, group D, member 1	transcription
109	mCG6431	mouse homolog of ZKR4	mouse homolog of ZKR4	ZKR4	zinc finger DNA binding protein ZER6	transcription
110	mCG15560	Rest	RE1-silencing transcription factor	REST	RE1-silencing transcription factor	transcription
111	mCG4503	Tbp	TATA box binding protein	TBP	TATA box binding protein	transcription

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Table 1 (continued)

Nr.	Cahera Gene	Mouse Gene Symbol	Mouse Gene Name	Human Gene Symbol	Human Gene Name	Group
112	mCG13054	Yy1	YY1 transcription factor	YY1	YY1 transcription factor	transcription
113	mCG14967	Zfp238	zinc finger protein 238	ZNF238	zinc finger protein 238	transcription
114	mCG33343	Zfp287	zinc finger protein 287	ZNF287	zinc finger protein 287	transcription
115	mCG12285	Zfp319	zinc finger protein 319	KIAA1386	KIAA1386	unknown
116	mCG15799	Lzrs2	leucine-rich repeat-containing 2	LRRC2	leucine-rich repeat-containing 2	unknown
117	mCG11132	Satb1	special AT-rich sequence binding protein 1	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-assoc...)	unknown
118	mCG53918	Slfn4	schlafen 4	unknown	unknown	unknown
119	mCG10290	unknown	unknown	unknown	unknown	unknown
120	mCG10613	unknown	unknown	unknown	unknown	unknown
121	mCG11224	unknown	unknown	unknown	unknown	unknown
122	mCG11325	unknown	unknown	unknown	unknown	unknown
123	mCG11359	unknown	unknown	unknown	unknown	unknown
124	mCG11803	unknown	unknown	unknown	unknown	unknown
125	mCG11817	unknown	unknown	unknown	unknown	unknown
126	mCG13566	unknown	unknown	unknown	unknown	unknown
127	mCG13630	unknown	unknown	unknown	unknown	unknown
128	mCG13824	unknown	unknown	unknown	unknown	unknown
129	mCG13346	unknown	unknown	unknown	unknown	unknown
130	mCG14143	unknown	unknown	unknown	unknown	unknown
131	mCG14155	unknown	unknown	unknown	unknown	unknown
132	mCG14342	unknown	unknown	unknown	unknown	unknown
133	mCG15141	unknown	unknown	unknown	unknown	unknown
134	mCG15227	unknown	unknown	unknown	unknown	unknown
135	mCG15743	unknown	unknown	unknown	unknown	unknown
136	mCG16959	unknown	unknown	unknown	unknown	unknown
137	mCG17127	unknown	unknown	unknown	unknown	unknown
138	mCG17140	unknown	unknown	unknown	unknown	unknown
139	mCG17143	unknown	unknown	unknown	unknown	unknown
140	mCG15447	unknown	unknown	unknown	unknown	unknown
141	mCG17569	unknown	unknown	unknown	unknown	unknown
142	mCG17731	unknown	unknown	unknown	unknown	unknown
143	mCG17799	unknown	unknown	unknown	unknown	unknown
144	mCG17902	unknown	unknown	unknown	unknown	unknown
145	mCG18918	unknown	unknown	unknown	unknown	unknown
146	mCG18034	unknown	unknown	unknown	unknown	unknown
147	mCG18196	unknown	unknown	unknown	unknown	unknown
148	mCG18663	unknown	unknown	unknown	unknown	unknown
149	mCG18737	unknown	unknown	unknown	unknown	unknown
150	mCG20276	unknown	unknown	unknown	unknown	unknown
151	mCG20905	unknown	unknown	unknown	unknown	unknown
152	mCG20994	unknown	unknown	unknown	unknown	unknown
153	mCG21405	unknown	unknown	unknown	unknown	unknown
154	mCG21505	unknown	unknown	unknown	unknown	unknown
155	mCG21529	unknown	unknown	unknown	unknown	unknown
156	mCG21530	unknown	unknown	unknown	unknown	unknown
157	mCG21803	unknown	unknown	unknown	unknown	unknown
158	mCG22074	unknown	unknown	unknown	unknown	unknown
159	mCG22045	unknown	unknown	unknown	unknown	unknown
160	mCG22336	unknown	unknown	unknown	unknown	unknown
161	mCG23253	unknown	unknown	unknown	unknown	unknown
162	mCG22772	unknown	unknown	unknown	unknown	unknown
163	mCG23022	unknown	unknown	unknown	unknown	unknown
164	mCG23035	unknown	unknown	unknown	unknown	unknown
165	mCG23069	unknown	unknown	unknown	unknown	unknown
166	mCG23075	unknown	unknown	unknown	unknown	unknown
167	mCG23310	unknown	unknown	unknown	unknown	unknown
168	mCG25543	unknown	unknown	unknown	unknown	unknown
169	mCG2824	unknown	unknown	unknown	unknown	unknown
170	mCG3347	unknown	unknown	unknown	unknown	unknown
171	mCG5031	unknown	unknown	unknown	unknown	unknown
172	mCG3729	unknown	unknown	unknown	unknown	unknown
173	mCG50409	unknown	unknown	unknown	unknown	unknown
174	mCG50451	unknown	unknown	unknown	unknown	unknown

Table 1 (continued)

9

Nr.	Column Gene	Mouse Gene Symbol	Mouse Gene Name	Human Gene Symbol	Human Gene Name	group
176	mCG5069	unknown	unknown	unknown	unknown	unknown
177	mCG1070	unknown	unknown	unknown	unknown	unknown
178	mCG51193	unknown	unknown	unknown	unknown	unknown
179	mCG52232	unknown	unknown	unknown	unknown	unknown
180	mCG53490	unknown	unknown	unknown	unknown	unknown
181	mCG53009	unknown	unknown	unknown	unknown	unknown
182	mCG53724	unknown	unknown	unknown	unknown	unknown
183	mCG53703	unknown	unknown	unknown	unknown	unknown
184	mCG55015	unknown	unknown	unknown	unknown	unknown
185	mCG55190	unknown	unknown	unknown	unknown	unknown
186	mCG53265	unknown	unknown	unknown	unknown	unknown
187	mCG55612	unknown	unknown	unknown	unknown	unknown
188	mCG56049	unknown	unknown	unknown	unknown	unknown
189	mCG56019	unknown	unknown	unknown	unknown	unknown
190	mCG56716	unknown	unknown	unknown	unknown	unknown
191	mCG57122	unknown	unknown	unknown	unknown	unknown
192	mCG57265	unknown	unknown	unknown	unknown	unknown
193	mCG57417	unknown	unknown	unknown	unknown	unknown
194	mCG57827	unknown	unknown	unknown	unknown	unknown
195	mCG58234	unknown	unknown	unknown	unknown	unknown
196	mCG58315	unknown	unknown	unknown	unknown	unknown
197	mCG58900	unknown	unknown	unknown	unknown	unknown
198	mCG58903	unknown	unknown	unknown	unknown	unknown
199	mCG58984	unknown	unknown	unknown	unknown	unknown
200	mCG59275	unknown	unknown	unknown	unknown	unknown
201	mCG59533	unknown	unknown	unknown	unknown	unknown
202	mCG59662	unknown	unknown	unknown	unknown	unknown
203	mCG59810	unknown	unknown	unknown	unknown	unknown
204	mCG59997	unknown	unknown	unknown	unknown	unknown
205	mCG60076	unknown	unknown	unknown	unknown	unknown
206	mCG60093	unknown	unknown	unknown	unknown	unknown
207	mCG61221	unknown	unknown	unknown	unknown	unknown
208	mCG61161	unknown	unknown	unknown	unknown	unknown
209	mCG61197	unknown	unknown	unknown	unknown	unknown
210	mCG61907	unknown	unknown	unknown	unknown	unknown
211	mCG61943	unknown	unknown	unknown	unknown	unknown
212	mCG62177	unknown	unknown	unknown	unknown	unknown
213	mCG62971	unknown	unknown	unknown	unknown	unknown
214	mCG63537	unknown	unknown	unknown	unknown	unknown
215	mCG63601	unknown	unknown	unknown	unknown	unknown
216	mCG64346	unknown	unknown	unknown	unknown	unknown
217	mCG64232	unknown	unknown	unknown	unknown	unknown
218	mCG64398	unknown	unknown	unknown	unknown	unknown
219	mCG65022	unknown	unknown	unknown	unknown	unknown
220	mCG65505	unknown	unknown	unknown	unknown	unknown
221	mCG65785	unknown	unknown	unknown	unknown	unknown
222	mCG66128	unknown	unknown	unknown	unknown	unknown
223	mCG66575	unknown	unknown	unknown	unknown	unknown
224	mCG66776	unknown	unknown	unknown	unknown	unknown
225	mCG66965	unknown	unknown	unknown	unknown	unknown
226	mCG67831	unknown	unknown	unknown	unknown	unknown
227	mCG67756	unknown	unknown	unknown	unknown	unknown
228	mCG8424	unknown	unknown	unknown	unknown	unknown
229	mCG9007	unknown	unknown	unknown	unknown	unknown
230	mCG8537	unknown	unknown	unknown	unknown	unknown
231	mCG3530	unknown	unknown	unknown	unknown	unknown
232	mCG9791	unknown	unknown	unknown	unknown	unknown
233	mCG9793	unknown	unknown	unknown	unknown	unknown
234	mCG9843	unknown	unknown	unknown	unknown	unknown
235	mCG9875	unknown	unknown	unknown	unknown	unknown
236	mCG9977	unknown	unknown	unknown	unknown	unknown
237	mCG9980	unknown	unknown	unknown	unknown	unknown

The first object of the present invention to provide novel genes involved in tumor development for use in the design of tumor specific therapies is thus achieved by using the human homologues of the murine genes of Table 1 to

5　develop inhibitors directed against these genes and/or their expression products and to use these inhibitors for the preparation of pharmaceutical compositions for the treatment of cancer.

The term "human homologue" as used herein should be

10　interpreted as a human gene having the same function as the gene identified in mouse.

In one embodiment of the present invention, the inhibitors are antibodies and/or antibody derivatives directed against the expression products of the genes listed

15　in Table 1. Such antibodies and/or antibody derivatives such as scFv, Fab, chimeric, bifunctional and other antibody-derived molecules can be obtained using standard techniques generally known to the person skilled in the art. Therapeutic antibodies are useful against gene expression products

20　located on the cellular membrane. Antibodies may influence the function of their target proteins by for example steric hindrance or blocking at least one of the functional domains of those proteins. In addition, antibodies may be used for deliverance of at least one toxic compound linked thereto to

25　a tumor cell.

In a second embodiment of the present invention, the inhibitor is a small molecule capable of interfering with the function of the protein encoded by the gene involved in tumor development. In addition, small molecules can be used for

30　deliverance of at least one linked toxic compound to a tumor cell.

Small molecule inhibitors are usually chemical entities that can be obtained by screening of already

existing libraries of compounds or by designing compounds based on the structure of the protein encoded by a gene involved in tumor development. Briefly, the structure of at least a fragment of the protein is determined by either

5 Nuclear Magnetic Resonance or X-ray crystallography. Based on this structure, a virtual screening of compounds is performed. The selected compounds are synthesized using medicinal and/or combinatorial chemistry and thereafter analyzed for their inhibitory effect on the protein in vitro

10 and in vivo. This step can be repeated until a compound is selected with the desired inhibitory effect. After optimization of the compound, its toxicity profile and efficacy as cancer therapeutic is tested in vivo using appropriate animal model systems.

15   The expression level of a gene can either be decreased or increased during tumor development. Naturally, inhibitors are used when the expression levels are elevated.

On a different level of inhibition nucleic acids can be used to block the production of proteins by destroying the

20 mRNA transcribed from the gene. This can be achieved by antisense drugs or by RNA interference (RNAi). By acting at this early stage in the disease process, these drugs prevent the production of a disease-causing protein. The present invention relates to antisense drugs, such as antisense RNA

25 and antisense oligodeoxynucleotides, directed against the genes listed in Table 1. Each antisense drug binds to a specific sequence of nucleotides in its mRNA target to inhibit production of the protein encoded by the target mRNA. The invention furthermore relates to RNAi molecules. RNAi

30 refers to the introduction of homologous double stranded RNA to specifically target the transcription product of a gene, resulting in a null or hypomorphic phenotype. RNA interference requires an initiation step and an effector

step. In the first step, input double-stranded (ds) RNA is processed into 21-23-nucleotide 'guide sequences'. These may be single- or double-stranded. The guide RNAs are incorporated into a nuclease complex, called the RNA-induced

5 silencing complex (RISC), which acts in the second effector step to destroy mRNAs that are recognized by the guide RNAs through base-pairing interactions. RNAi molecules are thus double stranded RNAs (dsRNAs) that are very potent in silencing the expression of the target gene. The invention

10 provides dsRNAs complementary to the genes listed in Table 1.

The invention relates further to gene therapy, in which the genes listed in Table 1 are used for the design of dominant-negative forms of these genes which inhibit the function of their wild-type counterparts following their

15 directed expression in a cancer cell.

A further aspect of the present invention relates to the use of the murine genes as well as their human homologues listed in Table 1 or their products for the development of reagents for diagnosis of cancers.

20 The invention also provides diagnostic compositions for diagnosing cancer, comprising histological examination of tissues specimens using specific antibodies directed against the products of the genes listed in Table 1 and/or in-situ hybridisation analysis of gene expression using specific RNA

25 probes directed these genes.

Another object of the present invention is to provide a pharmaceutical composition comprising the inhibitors according to the present invention as active ingredient for the treatment of cancer. The composition can further comprise

30 at least one pharmaceutical acceptable additive like for example a carrier, an emulsifier, or a conservative.

In addition, it is the object of the present invention to provide a method for treatment of cancer

patients which method comprises the administration of the pharmaceutical composition according to the invention to cancer patients.

The invention will be further illustrated in the

5 examples that follow and which are not given to limit the invention. Example 1 describes how the genes of Table 1 were identified. Example 2 describes the analysis of the YY1 gene with respect to its involvement in tumor development. Example 3 describes the development of inhibitors of the genes and

10 their encoded products.

Examples

EXAMPLE 1

15 **Identification of common viral insertion sites (CIS)**

INTRODUCTION

To identify common viral integration sites in mouse tumors and cell lines, the MuLV virus was used. Mice and cell

20 lines were either infected with murine leukemia virus 1.4 (Graffi-1.4 MuLV) or with Cas-Br-M MuLV.

The Graffi-MuLV is an ecotropic retroviral complex causing leukemias in mice. This viral complex does not contain oncogenic sequences itself but rather deregulates

25 genes due to proviral integrations. Graffi-1.4 MuLV is a subclone of this complex and predominantly induces myeloid leukemias.

NIH/Swiss mice infected with Cas-Br-M MuLV develop myeloid or lymphoid malignancies also as a result of

30 retroviral insertion that affect target genes.

MATERIALS AND METHODS

1. Induction of leukemias

Newborn FVB/N mice or NIH/Swiss were injected
5   subcutaneously with 100 µl of a cell culture supernatant of
Graffi-1.4 MuLV or Cas-Br-M MuLV producing NIH3T3 cells,
respectively. Mice were checked daily for symptoms of
illness, i.e., apathy, white ears and tail, impaired
interaction with cage-mates, weight-loss, and dull fur.
10   Typically, leukemic mice suffered from enlarged spleens,
livers, thymuses, and lymph nodes. From these primary tumors,
chromosomal DNA was isolated for PCR-based screening. Blood
samples were taken from the heart. For morphological
analysis, blood smears and cytospins were fixed in methanol,
15   May-Grünwald-Giemsa (MGG) stained and analyzed.

Single-cell suspensions of different organs were
analyzed by flow cytometry using a flow cytometer. The cells
were labeled with the following rat monoclonal antibodies:
ER-MP54 (ER-MP54), ER-MP58 (ER-MP58), M1/70 (Mac-1), F4/80
20   (F4/80), RB68C5 (GR-1), ER-MP21 (transferrin receptor),
TER119 (Glycophorin A), 59-AD2.2 (Thy-1), KT3 (CD3), RA3 6B2
(B220) and E13 161-7(Sca1). Immunodetection was performed
utilizing a Goat-anti-Rat antibody coupled to fluorescein
isothiocyanate.
25

2. Inverse PCR on Graffi-1.4 MuLV induced leukemias

Genomic DNA from the primary tumors was digested with
HhaI. After ligation, a first PCR was performed using Graffi-
1.4 MuLV (LTR) specific primers L1
30   (5' TGCAAGATGGCGTTACTGTAGCTAG 3') and L2
(5' CCAGGTTGCCCCAAAGACCTG 3') (cycling conditions were 1 min
at 94°C, 1 min at 65°C, 3 min at 72°C [30 cycles]). For the
second nested PCR the primers L1N

(5' AGCCTTATGGTGGGGTCTTTC 3') and L2N
(5' AAAGACCTGAAACGACCTTGC 3') (15 cycles) were used. The PCR
reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM
KCL, 1.5 mM $MgCl_2$, 200 μM dNTP's, 10 pmol of each primer and
5   2.5 units of Taq-polymerase. The PCR fragments were analyzed
on a 1% agarose gel and cloned in a TA cloning vector
according to standard procedures.

PCR products were sequenced with the M13 forward
primer 5' GACCGGCAGCAAAATG 3' and M13 reverse primer
10 5' CAGGAAACAGCTATGAC 3'. Virus flanking genomic sequences
were identified using the National Center for Biotechnology
Information (NCBI) and Celera databases.

3. **Inverse PCR and RT-PCR on Cas-Br-M MuLV leukemias**

15   Five μg of genomic DNA was digested with *Sau3A* or
with *SstI*. The products were treated with T4-ligase, which
resulted in the formation of circularized products.
Subsequently, an inverse PCR (ICPR) strategy was used with
primers specific for the Cas-Br-M MuLV LTR. For the *Sau3A*
20 digested/ligated fragments, the first PCR reaction was
carried out with primers pLTR4 (5'-CCG AAA CTG CTT ACC ACA-
3') and pLTR3
(5'-GGT CTC CTC AGA GTG ATT-3'), followed by a nested PCR
using pLTR5 (5'-ACC ACA GAT ATC CTG TTT-3') and pLTR6
25 (5'-GTG ATT GAC TAC CCG CTT-3'). Cycle conditions for both
PCRs were 15" at 94°C, 30" at 57°C, and 2' at 72°C for 10
cycles, and an additional 20 cycles following the conditions
15" at 94°C, 30" at 57°C, and 2'30" at 72°C. Reactions were
performed using Expand High Fidelity PCR System. In case of
30 *SstI* digested genomic DNA, the circularized DNA was amplified
using primers pLTR9 (5'-GAC TCA GTC TAT CGG AGG AC-3') and
pLTR1 (5'-CTT GCT GTT GCA TCC GAC TGG-3'), and pLTR10
(5'-GTG AGG GGT TGT GTG CTC-3') and 2

(5'-GTC TCG CTG TTC CTT GGG AGG-3'), respectively. The first
PCR was performed for 30 cycles 30" at 94°C, 1' at 60°C, and
3' at 72°C. The reaction was carried out with Expand High
Fidelity PCR system. Nested PCR conditions were 30 cycles of
5   30" at 94°C, 1' at 58°C, and 1' at 72°C. This reaction was
performed with Taq polymerase.

        For RT-PCR, total RNA was isolated through a CsCl
gradient. First strand cDNA was obtained by reverse
transcriptase (RT) reactions with an oligo(dT)-adapter primer
10 (5'-GTC GCG AAT TCG TCG ACG CG(dT)$_{15}$-3') at 37°C with 5 µg
RNA, using the Superscript™ Preamplification System.
Subsequently, PCRs (1' at 94°C, 1' at 58°C, 3' at 72°C (30
cycles)) were performed on the RT reactions of the leukemias
by using the LTR specific primer pLTR6 and the adapter primer
15 (5'-GTC GCG AAT TCG TCG ACG CG-3'). PCR products were
directly cloned into pCR2.1 and subjected to nucleotide
sequencing with the M13 forward primer 5'GACCGGCAGCAAAATG 3'
and m13 reverse primer 5'CAGGAAACAGCTATGAC 3'. Nucleotide
sequences were compared to the NCBI and Celera databases for
20 analysis.

Results
1. Graffi-1.4 MuLV induced leukemias
        Leukemias developed 4 to 6 months after subcutaneous
25 injection of newborn FVB/N mice with Graffi-1.4 MuLV. Forty-
eight of the 59 leukemias (81%) analyzed exhibited
morphological characteristics of myeloid cells. Blast cell
percentages in the bone marrow ranged from 24 to 90% with an
average of 48%. Leukemia cells expressed immunophenotypic
30 marker profiles consistent with their myeloid appearance,
e.g., ER-MP54+, ER-MP58+, CD3-, GR-1+. Six leukemias with
blast-like morphology showed no immunophenotypic
differentiation markers, suggesting that these tumors

represented very immature leukemias. Only 3 leukemias were of T-lymphoid origin (CD3+/MP58-/Thy1+) and 2 showed mixed myeloid and erythroid features (Ter119+/ER-MP58+/F4/80+). These results demonstrate that Graffi-1.4 MuLV infection
5   predominantly induce myeloid leukemia in FVB/N mice.

The provirus flanks were cloned, subjected to nucleotide sequencing, and blasted against the Celera and NCBI databases resulting in the identification of common insertion sites. Of the genes identified, the ones that were
10   so far not described to be involved in tumor development are listed in Table 1 combined with the novel cancer genes identified from Cas-Br-M MuLV induced leukemias.

2. Cas-Br-M MuLV induced leukemias

15   Cas-Br-M MuLV injected newborn NIH/Swiss mice developed leukemias by approximately 140 to 400 days postinoculation. Most of these were myeloid leukemias (59%), although T-cell (21%), and mixed T-cell/myeloid (21%) leukemias were found.

20   To clone viral integration sites, a virus-LTR (long terminal repeat) specific inverse PCR as well as RT-PCR were applied as complementary approaches using DNA or RNA from 35 myeloid leukemias. The inverse PCR method was carried out on 19 primary leukemias and 9 cell lines, whereas the RT-PCR
25   based technique was performed on 12 cell lines and 2 primary leukemias.

The provirus flanks were subjected to nucleotide sequencing, blasted against the Celera and NCBI databases resulting in the identification of common insertion sites. Of
30   the genes identified, the ones that were so far not described to be involved in tumor development are listed in Table 1 combined with the novel cancer genes identified from Graffi-1.4 MuLV induced leukemias.

DISCUSSION

Although proviral integrations occur randomly, they may affect the expression or function of nearby genes. If a gene is affected in two or more independent tumors, this

5   indicates that these integrations provide a selective advantage and therefore contribute to tumor development. Multiple of these common insertion sites were identified of which a large number are demonstrated for the first time to play a role in cancer. Importantly, several of the other

10 genes identified are well-known cancer genes validating the approach. This example shows that the pursued strategy can be successfully used to identify novel genes that are involved in tumor development.

15

EXAMPLE 2

Analysis of proviral integration sites in the YY1 gene and their effect on tumorigenisis

20 **INTRODUCTION**

YY1 is a transcription factor of the GLI-Krüppel zinc finger protein family that has been reported to activate or repress transcription of a large variety of cellular and viral genes. Additionally, YY1 regulates gene expression in a

25 cell-cycle dependent fashion. This may at least in part be due to a control mechanism involving the retinoblastoma protein (Rb), which releases YY1 in the S-phase of the cell cycle. The transcriptional activity of YY1 is positively regulated through acetylation of the protein by p300 and PCAF

30 and negatively regulated by deacetylation by histone deacetylases HDAC1, HDAC2 and HDAC3

This example describes that the YY1 gene is commonly targeted in Graffi-1.4 MuLV-induced leukemias and that

deregulation of *YY1* expression leads to defects in myeloid cell development and contributes to leukemogenesis.

MATERIALS AND METHODS

5 **1. Graffi-1.4 MuLV -induced leukemias**

Newborn FVB/N mice were injected subcutaneously with 100 μl of a cell culture supernatant of Graffi-1.4 MuLV - producing NIH3T3 cells. Mice were checked daily for symptoms of illness, i.e., apathy, white ears and tail, impaired

10 interaction with cage-mates, weight-loss, and dull fur. Typically, leukemic mice suffered from enlarged spleens, livers, thymuses, and lymph nodes. From these primary tumors, chromosomal DNA was isolated for PCR-based screening. Blood samples were taken from the heart. For morphological

15 analysis, blood smears and cytospins were fixed in methanol, May-Grünwald-Giemsa (MGG) stained and analyzed.

2. Immunophenotyping of the leukemic cells

Single-cell suspensions of different organs were

20 analyzed by flow cytometry using a flow cytometer. The cells were labeled with the following rat monoclonal antibodies: ER-MP54 (ER-MP54), ER-MP58 (ER-MP58), M1/70 (Mac-1), F4/80 (F4/80), RB68C5 (GR-1), ER-MP21 (transferrin receptor), TER119 (Glycophorin A), 59-AD2.2 (Thy-1), KT3 (CD3), RA3 6B2

25 (B220) and E13 161-7(Sca1). Immunodetection was performed utilizing a Goat-anti-Rat antibody coupled to fluorescein isothiocyanate.

3. Production of retroviral vectors

30 The plasmid pCMV-HA-YY1, containing hemagglutinin (HA)-tagged full length human YY1 cDNA, was digested with XbaI and ApaI, and the HA-YY1 fragment was blunted and ligated into the HpaI site of pLNCX and pBabe retroviral

vectors. PhoenixA packaging cells were transfected with pLNCX-HA-YY1 or pBABE-HA-YY1. Supernatants containing high-titer, helper-free recombinant virus were harvested from 80% confluent producer cells cultured for 16-20 hours in

5   Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum (FCS), penicillin (100 IU/ml) and streptomycin 100 ng/ml). To determine titers of BABE-HA-YY1 and BABE control virus, the virus particles were pelleted by ultracentrifugation at 41,000 rpm and RNA was extracted with

10 phenol (pH=4.0) and spot-blotted on nitrocellulose filters. This blot was hybridized with a BABE specific probe (SV40 fragment, BamHI-HindIII digest).

4. Cell culture and retroviral gene transfer

15   The interleukin-3 (IL-3) dependent murine myeloid cell line 32D containing the human wild type G-CSF- receptor (32D-WT1) was infected with pLNCX-HA-YY1 virus and selected with G418. Several independent clones were expanded for further analysis.

20   Hematopoietic cells were harvested from the femurs and tibiae of 8 to 12 week old FVB mice. After depletion of adherent cells, the remaining cells were fractionated on a Percoll™ gradient. Fraction I (density 1.058/1.0645) containing the earliest hematopoietic progenitor cells was collected.

25 Cells were washed twice in HBSS/5% FCS/0.5% BSA and then prestimulated for 2 days at a final concentration of $5x10^5$ cells/ml in Cell Gro® supplemented with a cytokine cocktail composed of mIL-3 (10 ng/ml), hFlt3-ligand, hTPO, mSCF (100 ng/ml) and GM-CSF (2 u/ml). Retroviral infection was performed

30 in culture dishes coated with the recombinant fibronectin fragment CH-296 at a concentration of 12 mg/ml. Before adding the bone marrow cells, the dishes were preincubated with virus supernatant (BABE-HA-YY1 or empty BABE) for 30 min at 37°C.

Subsequently, bone marrow cells were resuspended and mixed with fresh virus supernatant in a 1:1 ratio and a fresh cytokine cocktail was added. The cells ($5x10^5$) were cultured overnight at 37°C and 5% CO_2. Virus supernatant and cytokine cocktail

5   were refreshed again the next day and cells were cultured for another 24 hrs.

For colony assays, bone marrow cells were plated at densities of 1 to $5x10^4$ cells per ml per dish in triplicate in methylcellulose medium supplemented with 30% FBS, 1% BSA, 0.1mM

10   2-mercaptoethanol, 2mM L-glutamine, GM-CSF (20 u/ml) and with or without 2.5 mg/ml puromycin to evaluate the infection efficiency of the different retroviruses. Colonies consisting of more than 50 cells were counted on day 7 of culture.

15   5. **Promoter activity assay**

The YY1 promoter containing subclone pdSS4.5 of 124 was digested with restriction enzymes MluI and BglII. This YY1 promoter fragment was cloned in the luciferase reporter plasmid pGL3. The Graffi-1.4 MuLV LTR sequence was cloned in

20   both orientations in the HpaI site. The Sp1 site was mutated with the QuikChange Site-Directed Mutagenesis Kit by using primer SPIMUTF: 5' GGACGGTTCGGGGCGAGAGC 3' and primer SPIMUTR: 5' GCTCTCGCCCCGAACCGTCC 3'. As a positive control pRSV-Luc was used. The pRSV-β-galactosidase was used for

25   reference. Empty pGL3 vector served as a negative control. Luciferase assays were performed in HEK 293 cells according to standard procedures. Cells were transfected by $CaPO_4$ precititation with a mixture of 5 mg derived pGL3 plasmid containing different promoter sequences and 2.5 mg pRSV-β-

30   galactosidase, supplemented with empty vector to a total amount of 20 mg of total plasmid per ml.

6. **Inverse PCR on Graffi-1.4 MuLV induced leukemias**

Genomic DNA from the primary tumors was digested with HhaI. After ligation, a first PCR was performed using Graffi-1.4 MuLV (LTR) specific primers L1 (5′ TGCAAGATGGCGTTACTGTAGCTAG 3′) and L2

5   (5′ CCAGGTTGCCCCAAAGACCTG 3′) (cycling conditions were 1 min at 94°C, 1 min at 65°C, 3 min at 72°C [30 cycles]). For the second nested PCR the primers L1N (5′ AGCCTTATGGTGGGGTCTTTC 3′) and L2N (5′ AAAGACCTGAAACGACCTTGC 3′) (15 cycles) were used. The PCR

10   reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$, 200 µM dNTP's, 10 pmol of each primer and 2.5 units of Taq-polymerase. The PCR fragments were analyzed on a 1% agarose gel and cloned in the TA cloning vector according to standard procedures.

15

7. Detection of virus integration in the YY1 gene by specific nested PCRs

To determine the orientation and localization of the Graffi-1.4 provirus in the YY1 gene in an extended panel of

20   leukemias, a nested PCR was performed on 1 mg of genomic DNA from primary tumors. For the first PCR, YY1 promoter region specific primers Y1 5′ AGGAATCAGGAGCAGAAGAAAGTTTTGGGA 3′ and Y2 5' CAATAAAGTCTGCTCTGACGAGAAACGCC 3′ in combination with Graffi-1.4 MuLV LTR specific primers L1 and L2 were used.

25   Cycling conditions were 1 min 94°C, 1 min 65 °C, 3 min 72°C, 30 cycles. For the second PCR, the nested primers Y1N 5′ AAACTCTCTGACTTACCTCCCTCTCCAAAGA 3′ and Y2N 5' GTTCGTTTTGCCTTTACTCGTTACTCGGG 3′ in combination with the nested primers L1N and L2N (30 cycles) were used. The

30   obtained PCR products were analyzed by Southern blotting. To determine the orientation of the Graffi-1.4 provirus, the blots were hybridized with radiolabeled Y1P 5′ AAAACCTGCACAAGGACACCTTGCTAAGTATGTTT 3′ and Y2P 5′

AGCACACGGTCGGCTACGCTCCGTCCGCTACCGCA 3' at 45°C in Church
buffer (0.5 M phosphate buffer, pH 7.2, 7% (w/v) SDS, 10 mM
EDTA) overnight. Signals were visualized by autoradiography
according to standard procedures.

5

8. Nucleotide sequencing

PCR products were cloned in the TA cloning vector and
sequenced with the M13 forward primer 5' GACCGGCAGCAAAATG 3'
and M13 reverse primer 5' CAGGAAACAGCTATGAC 3'. Virus

10 flanking genomic sequences were identified using the National
Center for Biotechnology Information (NCBI) and Celera
databases.

9. Western blotting

15 Lysates of 32D cells were prepared and subjected to
Western blotting. Antibodies used to visualize YY1 were Goat-
anti-YY1 or rabbit anti-hemagglutinin (HA) for HA-tagged
YY11. Goat-anti-Actin was used to control for equal loading
of lysates.

20

RESULTS
1. Graffi-1.4 MuLV-induced leukemias

Leukemias developed 4 to 6 months after subcutaneous
injection of newborn FVB/N mice with Graffi-1.4 MuLV.

25 Forty-eight of the 59 leukemias (81%) analyzed exhibited
morphological characteristics of myeloid cells. Blast cell
percentages in the bone marrow ranged from 24 to 90% with an
average of 48%. Leukemia cells expressed immunophenotypic
marker profiles consistent with their myeloid appearance,

30 e.g., ER-MP54+, ER-MP58+, CD3-, GR-1+. Six leukemias with
blast-like morphology showed no immunophenotypic
differentiation markers, suggesting that these tumors
represented very immature leukemias. Only 3 leukemias were of

T-lymphoid origin (CD3+/MP58-/Thy1+) and 2 showed mixed
myeloid and erythroid features (Ter119+/ER-MP58+/F4/80+).
These results demonstrate that Graffi-1.4 MuLV infection
predominantly induce myeloid leukemia in FVB/N mice.

5

2. **Virus integrations in the YY1 gene**

Inverse PCR was used to identify genomic sequences
flanking Graffi-1.4 MuLV integrations. Integrations were
found in several genes previously demonstrated to be involved
10   in MuLV-induced leukemias, e.g., Notch-1, Nf1, p53, Fli-1 and
Evi-1, as well as in novel loci. One of these newly
identified integrations occurred in the YY1 locus,
approximately 0.8 Kb upstream of the transcriptional
initiation site. In a subsequent analysis on independent cell
15   samples using nested PCR with LTR and YY1 primers it was
found that 23 integrations were in the same region, i.e., 0.5
to 1.5 kb upstream of the transcriptional start site, in 14
out of 20 leukemias tested. Integrations occurred in both
orientations in an approximately equal ratio. Searches for
20   virus integrations in other parts of the gene using
appropriate primer sets were negative.

3. **Integration of Graffi-1.4 MuLV LTR deregulates YY1 transcription**

25   Because integration of the virus occurred in both
orientations, it is most likely that viral enhancer sequences
in the LTR cause increased YY1 transcription in these
leukemias. However, integration in the promoter region of a
gene does not always result in alterations in expression. To
30   study how virus integration influences YY1 expression, the
Graffi-1.4 LTR was introduced at position -1417 in the YY1
promoter and transcriptional activity using a transient
reporter assay was quantified. A two-fold higher YY1 promoter

activity was measured following insertion of the Graffi-1.4
MuLV LTR, irrespective of the orientation of the LTR. Because
a Sp1 binding site at position -48 to -39 has previously been
shown to be important for induction of YY1 transcription, the
5   effects of insertion of the Graffi-1.4 MuLV LTR after
disruption of this site were also studied. While mutation of
the Sp1 binding site resulted in a 50% reduction of normal
promoter activity, the enhanced promoter activity caused by
the integration of the LTR sequence was not affected. This
10   result demonstrates that virus integration causes enhanced
and Sp1-independent transcription of YY1.

4.   **Ectopic expression of YY1 blocks G-CSF-induced
neutrophilic differentiation of 32D cells**
15   The observation that integration of Graffi MuLV LTR
alters the expression of YY1 in murine acute myeloid leukemia
raised the question whether perturbed YY1 expression might
affect myeloid cell differentiation. 32D cell clones
expressing human G-CSF receptors (32D-WT1 cells) were
20   developed as a model to study neutrophilic differentiation.
Endogenous YY1 levels are high in parental 32D-WT1
cells cultured in IL-3 containing medium, under which
conditions the cells remain immature. However, upon transfer
of the cells to G-CSF-containing medium, in which cells
25   undergo neutrophilic differentiation, YY1 protein levels
declined from day 2 onward and remained low for the entire
culture period. To determine the consequences of perturbed
YY1 expression for myeloid cell development, we ectopically
expressed HA-tagged YY1 in these cells. As expected, YY1
30   expression in 32D-HA-YY1 cells remained high after switching
the cells to G-CSF. Growth rate and morphology of 32D-HA-YY1
cells cultured in IL-3 containing medium were similar to
nontransduced control cells. Strikingly, when 32D-HA-YY1

cells were switched to G-CSF-containing medium, neutrophilic differentiation was markedly reduced. Instead, cells with a blast-like morphology expanded exponentially for 8 days in these cultures and rapidly died thereafter as a result of

5 apoptosis. Thus, sustained *YY1* expression prevented G-CSF-induced myeloid differentiation of 32D cells without affecting the apoptotic response that normally accompanies G-CSF-induced differentiation.

10 **5. Ectopic expression of YY1 blocks CFU-GM colony formation**

The above-mentioned experiments show that constitutive expression of *YY1* interferes with the G-CSF-induced myeloid differentiation in 32D cells. Next, it was investigated how perturbed expression of *YY1* affects the

15 outgrowth of primary myelomonocytic progenitor cells (CFU-GM). To this end, mouse bone marrow cells were retrovirally transduced with BABE-HA-YY1 or empty BABE virus and plated in GM-CSF-containing colony assays. Notably, control bone marrow cells were subjected to equivalent titers

20 of control virus to exclude that differences in colony outgrowth were caused by variations in transduction efficiencies. Strikingly, GM-CSF-induced colony formation by HA-YY1-transduced bone marrow cells was almost completely blocked. This result demonstrates that perturbed expression

25 of *YY1*, instead of interfering with differentiation, causes a growth arrest or results in premature apoptosis of CFU-GM or their direct progeny.

DISCUSSION

30 This example demonstrates that the gene encoding the transcriptional regulator YY1 is located in a common virus integration site in Graffi-1.4 MuLV-induced myeloid leukemia. The integrations occurred exclusively in the 5' promoter

region of the gene, 0.5 to 1.5 kb upstream of the major transcriptional start site and resulted in a two-fold higher transcription of the YY1 gene.

Perturbed expression of YY1 inhibited G-CSF-induced
5 myeloid differentiation in 32D cells and prevented the clonal outgrowth of CFU-GM progenitor cells. Importantly, it was observed that integration of Graffi-virus LTR sequences not only enhanced *YY1* transcription, but also made it entirely independent of the recognition site for the transcription
10 factor Sp1, which has been shown to play a pivotal role in *YY1* expression. Although Sp1 expression has been reported to be high in hematopoietic cells, it is downregulated during differentiation in many cell types and may be involved in the decrease in YY1 protein levels observed during myeloid
15 differentiation. Therefore, bypass of Sp1 transcriptional control might be one of the mechanisms by which viral integration deregulates YY1 protein levels in leukemic cells.

By demonstrating that perturbed expression of YY1 due to proviral integrations contributes to tumor development,
20 this example validates the strategy of identifying novel cancer genes by retroviral insertional tagging.

EXAMPLE 3

<u>Preparation of inhibitors of the expression products of gene</u>s
25 <u>involved in cancer from Examples 1 and 2</u>

Confirmation of the involvement of the identified genes in primary human tumors

The expression of the described genes, that were
30 originally identified by genome-wide functional screens involving retroviral insertional tagging in mouse models (see Examples 1 and 2), is determined in a panel of different human tumor samples using microarray analysis. From an

extensive set of primary human tumors of various organs, RNA is prepared using standard laboratory techniques to investigate the expression of the described genes in these samples relative to their expression in normal, unaffected

5 tissues from the same origin by using microarrays on which these genes, or parts thereof, are spotted. Microarray analysis allows rapid screening of a large set of genes in a single experiment (DeRisi et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer. Nat Genet

10 14:457-60, 1996; Lockhart et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 14:1649, 1996). Genes that are differentially expressed in tumor and normal tissues as determined by microarray analysis are further examined by other techniques

15 such as RT-PCR, Northern blot analysis, and, if gene-specific antibodies are available, Western blot analysis. Confirmation of differential expression of these genes demonstrates their involvement in human tumors. These findings are further substantiated by similar experiments using a panel of human

20 tumor cell lines.

Use of the identified genes as diagnostic tools for cancer
Genes that are differentially expressed in human tumors are used as diagnostic tools for cancer (according to

25 Welsh et al. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc Natl Acad Sci USA 98:1176-81, 2001). For example, certain genes are highly expressed in early-stage tumor cells compared with

30 normal tissue. In a clinical setting, the expression of these genes is determined and used as a marker for the (pre-) malignant status of tissue samples according to Hegde et al. (Identification of tumor markers in models of human

colorectal cancer using a 19,200-element complementary DNA microarray. Cancer Res 61:7792-7, 2001).

Functional importance of the identified genes for human cancer

Subsequently, the functional importance of the differentially expressed genes for tumor cells is determined by over-expression as well as by inhibition of the expression of these genes. Selected human tumor cell lines are transfected either with plasmids encoding cDNA of the genes or with plasmids encoding RNA interference probes for the genes. RNA interference is a recently developed technique that involves introduction of double-stranded oligonucleotides designed to block expression of a specific gene (see Elbashir et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494-8, 2001; Brummelkamp et al. A system for stable expression of short interfering RNAs in mammalian cells. Science 296:550-3, 2002). Using standard laboratory techniques and assays, the transfected cell lines are extensively checked for altered phenotypes that are relevant for the tumor cells, e.g. cell cycle status, proliferation, adhesion, apoptosis, invasive abilities, etc. These experiments demonstrate the functional importance of the identified genes for human cancer.

Structure-function analysis of selected targets

Genes that are shown to be both differentially expressed and functionally important for human tumors are selected for structure-function analysis. Deletion and point mutation mutants of these genes are constructed and tested for their functional competence compared with wild-type genes (according to Ibanez et al. Structural and functional domains

of the myb oncogene: requirements for nuclear transport, myeloid transformation, and colony formation. J Virol 62:1981-8, 1988; Rebay *et al.* Specific truncations of Drosophila Notch define dominant activated and dominant

5 negative forms of the receptor. Cell 74:319-29, 1993). These studies lead to the identification of functionally critical domains as well as to dominant-negative variants, i.e. mutant genes that suppress the function of their endogenous counterparts (e.g. Kashles *et al.* A dominant negative

10 mutation suppresses the function of normal epidermal growth factor receptors by heterodimerization. Mol Cell Biol 11:1454-63, 1991). These dominant-negatives provide additional proof for the functional importance of the genes and give insight into which therapeutic approaches can be

15 pursued to interfere with their function. Furthermore, the cellular localization of the proteins encoded by the genes is determined by immunofluorescence using standard laboratory techniques. If the proteins are expressed on the cell-surface, this allows for the development of antibody-based

20 inhibitors.

Development of antibody-based inhibitors

Differentially expressed genes that encode membrane-bound proteins are selected as targets for conventional

25 antibody-based therapies. Antibodies are generated against functionally relevant domains of the proteins and subsequently screened for their ability to interfere with the target's function using standard techniques and assays (Schwartzberg. Clinical experience with edrecolomoab: a

30 monoclonal antibody therapy for colorectal carcinoma. Crit Rev Oncol Hematol 40:17-24, 2001; Herbst *et al.* Monoclonal antibodies to target epidermal growth factor receptor-positive tumors: a new paradigm for cancer therapy. Cancer

94:1593-611, 2002). These antibodies are also useful as diagnostic reagents (Syrigos et al. Use of monoclonal antibodies for the diagnosis and treatment of bladder cancer. Hybridoma 18:219-24, 1999).

Development of small molecule inhibitors

Differentially expressed genes that do not encode membrane-bound proteins are selected as targets for the development of small molecule inhibitors. To identify putative binding sites or pockets for small molecules on the surface of the target proteins, the three-dimensional structures of those targets are determined by standard crystallization techniques (de Vos et al. Three-dimensional structure of an oncogene protein: catalytic domain of human c-H-ras p21. Science 239:888-93, 1988; Williams et al. Crystal structure of the BRCT repeat region from the breast cancer-associated protein BRCA1. Nat Struct Biol 8:838-42, 2001). Additional mutational analysis is performed as mentioned above to confirm the functional importance of the identified binding sites. Subsequently, Cerius2 (Molecular Simulations Inc., San Diego, CA, USA) and Ludi/ACD (Accelrys Inc., San Diego, CA, USA) software is used for virtual screening of small molecule libraries (Bohm. The computer program Ludi: A new method for the de novo design of enzyme inhibitors. J Comp Aided Molec Design 6:61-78, 1992). The compounds identified as potential binders by these programs are synthesized by combinatorial chemistry and screened for binding affinity to the targets as well as for their inhibitory capacities of the target protein's function by standard in vitro and in vivo assays. In addition to the rational development of novel small molecules, existing small molecule compound libraries are screened using these assays to generate lead compounds. Lead compounds identified are

subsequently co-crystallized with the target to obtain
information on how the binding of the small molecule can be
improved (Zeslawska *et al.* Crystals of the urokinase type
plasminogen activator variant beta(c)-uPAin complex with
5   small molecule inhibitors open the way towards structure-
based drug design. J Mol Biol 301:465-75, 2000). Based on
these findings, novel compounds are designed, synthesized,
tested, and co-crystallized. This optimization process is
repeated for several rounds leading to the development of a
10   high-affinity compound of the invention that successfully
inhibits the function of its target protein. Finally, the
toxicity of the compound is tested using standard assays
(commercially available service via MDS Pharma Services,
Montreal, Quebec, Canada) after which it is screened in an
15   animal model system.

Development of antisense molecule inhibitors
        These inhibitors are either antisense RNA or antisense
oligodeoxynucleotides (antisense ODNs) and are prepared
20   synthetically or by means of recombinant DNA techniques. Both
methods are well within the reach of the person skilled in
the art. ODNs are smaller than complete antisense RNAs and
have therefore the advantage that they can more easily enter
the target cell. In order to avoid their digestion by DNAse,
25   ODNs but also antisense RNAs are chemically modified. For
targeting to the desired target cells, the molecules are
linked to ligands of receptors found on the target cells or
to antibodies directed against molecules on the surface of
the target cells.
30

Development of RNAi molecule inhibitors
        Double-stranded RNA corresponding to a particular gene
is a powerful suppressant of that gene. The ability of dsRNA

to suppress the expression of a gene corresponding to its own
sequence is also called post-transcriptional gene silencing
or PTGS. The only RNA molecules normally found in the
cytoplasm of a cell are molecules of single-stranded mRNA. If

5   the cell finds molecules of double-stranded RNA, dsRNA, it
uses an enzyme to cut them into fragments containing 21-25
base pairs (about 2 turns of a double helix). The two strands
of each fragment then separate enough to expose the antisense
strand so that it can bind to the complementary sense

10   sequence on a molecule of mRNA. This triggers cutting the
mRNA in that region thus destroying its ability to be
translated into a polypeptide. Introducing dsRNA
corresponding to a particular gene will knock out the cell's
endogenous expression of that gene. This can be done in

15   particular tissues at a chosen time. A possible disadvantage
of simply introducing dsRNA fragments into a cell is that
gene expression is only temporarily reduced. However, a more
permanent solution is provided by introducing into the cells
a DNA vector that can continuously synthesize a dsRNA

20   corresponding to the gene to be suppressed. RNAi molecules
are prepared by methods well known to the person skilled in
the art.

Efficacy of antibody-based and small molecule inhibitors in
25   **animal model systems**

The efficacy of both the antibody-based and small
molecule inhibitors are tested in an appropriate animal model
system before entry of these inhibitors into clinical
development (e.g. Brekken *et al.* Selective inhibition of

30   vascular endothelial growth factor (VEGF) receptor 2
(KDR/Flk-1) activity by a monoclonal anti-VEGF antibody
blocks tumor growth in mice. Cancer Res 60:5117-24, 2000;
Wilkinson *et al.* Antibody targeting studies in a transgenic

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murine model of spontaneous colorectal tumors. Proc Natl Acad
Sci USA 98:10256-60, 2001; Laird et al. SU6668 inhibits Flk-
1/KDR and PDGFRbeta in vivo, resulting in rapid apoptosis of
tumor vasculature and tumor regression in mice. FASEB J
5   16:681-90, 2002).

CLAIMS

1. Use of inhibitors of the expressed proteins of the murine genes and/or their human homologues listed in Table 1 for the preparation of a therapeutical composition for the treatment of cancer, in particular for the treatment of solid
5 tumors of lung, colon, breast, prostate, ovarian, pancreas and leukemia.

2. Use as claimed in claim 1, wherein the inhibitors are antibodies or derivatives thereof directed against the
10 expression products of the genes that are expressed on the cell membrane.

3. Use as claimed in claim 2, wherein the derivatives are selected from the group consisting of scFv fragments, Fab
15 fragments, chimeric antibodies, bifunctional antibodies, and other antibody-derived molecules.

4. Use as claimed in claim 1, wherein the inhibitors are small molecules interfering with the biological activity
20 of the protein expressed by the gene.

5. Use of inhibitors of the mRNA transcripts of the genes listed in Table 1 for the preparation of a therapeutical composition for the treatment of cancer.
25

6. Use as claimed in claim 5, wherein the inhibitors are antisense molecules, in particular antisense RNA or antisense oligodeoxynucleotides.

30   7. Use as claimed in claim 5, wherein the inhibitors are double stranded RNA molecules for RNA interference.

8. Use as claimed in claim 1 and claim 5, wherein the treatment comprises gene therapy.

9. Use as claimed in any one of the claims 1-8,
5   wherein the therapeutical composition is for treatment of inflammatory diseases.

10. Use of the murine genes and/or their human homologues listed in Table 1 or their expression products for
10 the diagnosis of cancer.

11. Use as claimed in claim 10, wherein the diagnosis is performed by means of histological analysis of tissue specimens using specific antibodies directed against gene
15 products, and/or in-situ hybridisation analysis of gene expression levels in tissue specimens using specific RNA probes directed against gene sequences.

12. Inhibitor compound directed against the expressed
20 proteins of a murine gene and/or its human homologue listed in Table 1 for use in the treatment of cancer.

13. Inhibitor compound as claimed in claim 12, which is an antibody or derivative thereof directed against the
25 expression products of a gene that is expressed on the cell membrane.

14. Inhibitor compound as claimed in claim 13, wherein the derivative is selected from the group consisting of scFv
30 fragments, Fab fragments, chimeric antibodies, bifunctional antibodies, or other antibody-derived molecules.

15. Inhibitor compound as claimed in claim 12, which is a small molecule interfering with the biological activity of the protein expressed by the gene.

5   16. Inhibitor compound directed against the transcription product (mRNA) of a murine gene and/or its human homologue listed in Table 1 for use in the treatment of cancer.

10   17. Inhibitor compound as claimed in claim 16, which is an antisense molecule, in particular an antisense RNA or an antisense oligodeoxynucleotide.

18. Inhibitor compound as claimed in claim 17, which
15 is a double stranded RNA molecule for RNA interference.

19. Diagnostic agent for diagnosing cancers in which one or more of the murine genes and/or their human homologues listed in Table 1 are involved.
20

20. Diagnostic agent as claimed in claim 19, which is a specific antibody directed against a gene product or a specific RNA probe specific for a gene sequence.

25   21. Therapeutical composition for the treatment of cancers in which one or more of the murine genes and/or their human homologues listed in Table 1 are involved, comprising a suitable excipient, carrier or diluent and one or more inhibitor compounds as claimed in claims 12-18.
30

22. Diagnostic composition for the diagnosis of cancers in which one or more of the murine genes and/or their human homologues listed in Table 1 are involved, comprising a

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suitable excipient, carrier or diluent and one or more
diagnostic compounds as claimed in claims 19-20.

5   23. Compositions as claimed in claim 21 or 22, wherein
the cancer is a solid tumor of e.g. lung, colon, breast,
prostate, ovarian, pancreas and leukemia.

       24. Method for the development of therapeutic
inhibitor compounds as claimed in claims 12-18, which method
10 comprises the steps:
            a) identification of genes involved in cancer, in
            particular by using retroviral insertional
            tagging, optionally in a specific genetic
            background;
15   b) validation of one or more of the identified
            genes as potential target gene(s) for the
            therapeutic compound by one or more of the
            following methods:
            c) confirmation of the identified gene by Northern
20   Blot analysis in cancer cell-lines;
            d) determination of the expression profile of the
            identified gene in tumors and normal tissue;
            e) determination of the functional importance of
            the identified genes for cancer;
25   f) production of the expression product of the
            gene; and
            g) use of the expression product of the gene for
            the production or design of a therapeutic
            compound.
30

       25. Method as claimed in claim 24, wherein the gene
identified in step a) is selected from the murine genes
and/or their human homologues listed in Table 1.

ABSTRACT

The invention relates to the use of inhibitors of the expressed proteins of the murine genes and/or their human homologues listed in Table 1 for the preparation of a therapeutical composition for the treatment of cancer, in particular for the treatment of solid tumors of lung, colon, breast, prostate, ovarian, pancreas and leukemia and the use of the genes listed in Table 1 for the diagnosis of cancer. The invention also relates to the therapeutical compositions comprising the inhibitors and to methods for development of the inhibitor compounds.

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